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**REGULATION OF THE *ASPERGILLUS NIDULANS*  
CYTOCHROME C GENE**

A thesis presented in partial fulfilment of  
the requirements for the degree of  
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at Massey University, Palmerston North  
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## **ABSTRACT**

The filamentous fungus *Aspergillus nidulans* has been genetically and biochemically well-characterised and thus provides an attractive model for studies on the regulation of eukaryotic gene expression. This study was undertaken to investigate the factors affecting respiratory function in *A. nidulans*. Due to the central role of cytochrome *c* in oxidative respiration, this study was focused on the cytochrome *c* gene and primarily upon how oxygen availability affects its expression.

The *Aspergillus nidulans* cytochrome *c* gene (*cycA*) appears to be transcriptionally activated in response to oxygen availability (Raitt *et al.*, 1994). In the yeast *S. cerevisiae*, oxygen availability activates its cytochrome *c* genes via a heme-activated protein HAP1, which binds to the promoter region of each gene (Pfiefer *et al.*, 1989). Since heme is only synthesised in the presence of oxygen, activity of the HAP1 protein serves as an intracellular signal of oxygen availability.

In the upstream region of the *cycA* gene, a sequence with homology to the *S. cerevisiae* HAP1 binding site was present (Raitt, 1992). To determine the significance of the putative HAP1 binding site and the role of other promoter sequences in the *A. nidulans* *cycA* gene, a promoter-reporter vector was constructed. However, upon sequencing of the *cycA* promoter in the reporter vector, a sequencing error was discovered in the published *cycA* gene by Raitt *et al.* (1994) which affected the position of the major translational start site. Further examination of the *cycA* sequence also revealed a possible undetected intron (Intron I). To determine the number of introns in the *cycA* gene, RT-PCR was performed on *cycA* RNA. Sequencing of the RT-PCR amplified products showed that the previously undetected intron (Intron I) was present, and that the *cycA* gene contains three instead of two introns as published by Raitt *et al.* (1994). Since the published ATG start site was located within Intron I, a new translational start site was proposed. The major consequences of these changes to the *cycA* gene was that the putative HAP1 site was now located within the coding region of the gene, and therefore could not be a regulatory element. In addition only 247 bp of *cycA* promoter sequence remained cloned for analysis. To obtain additional promoter sequence, an *A. nidulans* genomic library was screened with a *Bam*HI cDNA probe containing 224 bp of the 5' region of the *cycA* gene. Three positive clones were obtained, of which  $\lambda$ LM9 and  $\lambda$ LM5 were identical, and  $\lambda$ LM19 was an overlapping clone with  $\lambda$ LM9 and  $\lambda$ LM5. Restriction enzyme and Southern blott analysis of the two overlapping *cycA* clones, revealed that 2.1 kb *Eco*RI fragments from both clones contained the 5' region. The 2.1 kb *Eco*RI fragment from  $\lambda$ LM9 was cloned into pUC18 and sequenced. The completed

upstream sequence of the *A. nidulans* cytochrome *c* gene was obtained, and putative regulatory signals including the HAP1 binding site were found, and compared with published promoter sequences which regulate the expression of respiratory-encoding genes from yeast.

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